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UNITED STATES DEPARTMENT OF AGRICULTURE

BUREAU OF ENTOMOLOGY

FOREST INSECT INVESTIGATIONS

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CRITICAL LOW TEMPERATURES FOR THE WESTERN PINE AND MOUNTAIN PINE BEETLES

PROGRESS REPORT OF LABORATORY STUDIES

1933-34

by

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CRITICAL LOW TEMPERATURES FOR THE WESTERN PINE BEETLE AND MOUNTAIN PINE BEETLE

PROGRESS REPORT - LABORATORY STUDIES 1933 - 34.

INTRODUCTION

The widespread effects of low temperatures upon barkbeetle population during the winter of 1932-33 brought into the foreground the importance of cold as a factor in the natural control of these insects. Reports upon the results of this freeze, as shown by mortality of overwintering broods, were submitted by Keen, Beal, Salman and Miller during the spring of 1933. These studies confirmed the original supposition, based upon weather reports, that the western pine beetle had been adversely affected by the freeze throughout the ponderosa pine belt in eastern Washington, eastern Oregon and northeastern California; and that the mortality in many parts of this area had been extremely high. During the summer of 1933, field studies by Mr. Evenden and his assistants brought out the fact that the cold wave had also produced a high kill of the mountain pine beetle in various parts of the northern Rocky mountain region. From various sources it was shown that the kill of barkbeetles by low temperatures during this particular winter was unquestionably widely distributed, and that apparently the mortality far exceeded that for any previous winter of which we have record.

Because of the striking results of the 1932-33 winter kill shown by these studies, Dr. Craighead suggested that the western field laboratories of the Bureau of Entomology get together, review the information that had become available, and outline a program for further studies. This conference was held at Portland, Dec. 11-23, 1933, and an abstract of the discussions submitted.

At the Portland conference, it was agreed that low temperatures as a limiting factor of barkbeetle populations had been sufficiently demonstrated to warrant further study of the problem. It was evident that the opportunity to secure further data from the field would depend upon weather conditions which could not be foreseen, as a similar set of low temperatures and accompanying conditions like those of the winter of 1932-33 might not occur again for many years. For this reason, an immediate laboratory program was decided upon to test out the effect of temperatures under controlled conditions. The following points were set up as the objective of this laboratory work.

1. Determine the lethal points for all brood stages and at different seasonal periods for each of the more important barkbeetle species, working first on material from California localities.

2. Determine whether there are regional differences in

cold resistance for the same species due to development of race tendencies under different climatic conditions.

3. Determine whether the lethal points differ according to host for the same species, such as the mountain pine beetle which attacks white pine, sugar pine and lodgepole.

It was considered that information on these points will be of value in the interpretation of field data, when the occasion again arises to study the results of a killing cold wave.

An agreement was reached that the laboratory program should be centered at Berkeley until the advantages of some other location could be shown. This was due to the fact that some preliminary laboratory work had been undertaken by the personnel at this station, and the laboratory facilities and equipment at Berkeley were better developed than at the other stations. The Coeur d'Alene and Portland offices agreed to cooperate in this work to the extent of collecting and sending brood material to Berkeley for the experiments.

PROGRESS OF THE EXPERIMENTAL WORK

1. Program followed during 1933-34.

During the winter of 1933-34, a series of experiments were carried on at Berkeley under the supervision of the writer. G. R. Struble and Dr. A. Gordon assisted on the project, and the services of an Assistant to Technician, Gordon L. Smith, were available throughout a large part of the study. Shipments of material from the Sierra, Modoc, Ochoco and Coer d'Alene National Forests were used for western pine beetle studies; mountain pine beetle material was received from the Sierra National Forest, Crater Lake Park, Coeur d'Alene and Dillon, Montana.

A few tests of western pine beetle summer brood were made during the summer of 1934.

In September, 1934, arrangements were made with the University of California to assign a graduate student to the project on half time, under a special state fund, and Mr. J. C. Yuill, a graduate student in entomology, was assigned. Assistants, mostly students working on an hourly basis, were employed from time to time when needed for the tedious work of collecting larvae from the bark and making mortality counts.

2. Development of Apparatus.

Considerable attention was given by Mr. Yuill during the fall of 1934 to perfecting the refrigeration apparatus, and the electro-thermal equipment for measuring temperatures. Refrigeration equipment available at Berkeley for the project consisted of controlled temperature rooms

belonging to the University of California in the basement of Hilgard Hall. One of these, which was held at a temperature of 36° F. was used for storage of brood material; another room, held at a constant temperature of °F was used for certain tests of D.b. larvae at this point.

The Bureau of Entomology provided a small Frigidaire unit with two compartments of the well type, which was capable of developing temperatures down to -20° Fahr. In previous work it had been found that temperatures in these compartments varied according to vertical depth, due to stratification of air. This was corrected by installing a fan on the compartment lid, which developed rapid air circulation and insured equal temperatures at all points. (Photo No. 5)

A mercury switch and relay, connected with a U-tube thermo-regulator, was installed on the Frigidaire unit, which made it possible to set the machine for any given temperature and maintain it indefinitely. A flexible cable was installed which permitted setting of the thermo-regulator from the outside of the cabinet. The 20 thermocouples used for determining temperatures inside the cabinet were re-calibrated and the control panel rewired. By these changes, the accuracy and depend-ability of the equipment were greatly increased. (Photo No. 4.)

The experiments which have been run since December, 1933, were centered mainly upon two barkbeetle species, D. brevicomis and D. monticolae. Because of the wide differences in cold resistance and other responses shown by these two species, the results are presented and discussed separately.

DEFINITION OF TERMS USED IN DISCUSSION OF LOW LETHAL TEMPERATURES

Throughout this report specific terms are used in describing the effects of lethal temperatures and to prevent confusion it seems best to define them.

Temperature points. The minimum temperature to which the body of the insect is chilled. During the process of cooling, the insect may be exposed to all temperatures above this point, but no lower temperatures are reached.

Mortality percent. The percent which dead individuals compose of the total number of individuals in a group. If out of 100 individuals, 80 are killed at a critical point, the mortality is 80%.

Critical points. Temperatures at which mortality due to cold occurs. This applies to all points where killing is perceptible, whether the mortality resulting is partial or complete.

Freezing points. Temperatures at which the body of the insect becomes frozen due to crystallization of the free water, but does not result in death. This point may occur from 5 to 10° above the critical points.

Critical Range. The entire range of temperatures in which mortality due to cold occurs, from the highest point at which slight mortality develops, to the lowest point where 100 percent mortality first occurs.

Initial Critical Point. The highest point in the critical range at which mortality can be detected.

Ultimate Critical Point. The lowest point in the critical range below which no individuals survive.

Intermediate critical points. The temperatures between the initial and ultimate points. The mortality which may occur within a group of individuals at any of these points is variable, but never complete.

WESTERN PINE BEETLE STUDIES

Methods of Transporting and Handling Material.

Transportation of brood material from field to laboratory is a very simple matter for this species after the larvae reach the outer bark. Bark can be removed from log and broods shipped in their natural habitat. Very little, if any, abnormal mortality occurs, as long as extreme temperatures are not encountered. Such material can be stored for long periods at 30 to 40° F. without loss. Advanced larvae, pupae and new adults can be secured at any time by bringing the bark into favorable developmental temperatures around 70° F. and rearing them.

Containers used for Exposure to Critical Temperatures.

Brood in Outer Bark.

It is entirely feasible to expose larvae to low temperatures by placing bark sections in the refrigeration cabinets. Mortality counts are made after exposure by cutting larvae out of the bark. This method gives little opportunity for selection of material, however, as the number of individuals and their condition of development cannot be definitely determined until after exposure.

Repeated tests have shown that the immediate effects of critical temperatures are the same, whether exposure is made while larvae are in the bark, or after they have been removed and exposed naked in the compartment. It has been found that the use of naked larvae for exposure tests has several advantages over the bark method, as it is possible to make a selection of the material, so as to use uniform broods. It is also possible to determine mortality immediately after exposure and at any selected intervals thereafter.

When naked larvae are used for tests, they can be grouped in units of any number, but groups of 50 or 100 have been found to be the most convenient. It is necessary to keep these groups in some sort of a container during the entire period of exposure, and also during the subsequent period while under observation to determine the amount of mortality. Three types of containers have been used for this purpose.

Pasteboard boxes.

Small pasteboard boxes of the pill box type are serviceable. The chief objection is that unless the larvae are isolated in individual cells, some injury may result in handling, due to the tendency to roll around in the box. (Photo No. 1).

Paraffin cells.

A petri dish filled with paraffin is stamped with a mould, so as to form 50 open shallow cells and has given good results. This gives effective isolation and each larvae can be identified in follow-up observations. In most of the tests run during winter of 1933-34, this type of container was used. (Photo No. 2).

Bark Cells.

A plate of ponderosa pine bark is split and 50 small holes drilled to form cells. This gives complete isolation and as near to natural environmental conditions as possible, after larvae have been taken out of their original mines. (Photo No. 3).

In comparing results found in paraffin and bark cells, it was found there was some slight differences in the mortality under critical temperatures, but no tendencies could be recognized that were consistent. Such differences as were found could be accounted for by individual variations in cold resistance in the units of material. Apparently either type of container has no deleterious effect upon living larvae and pupae and each will permit the full effects of critical temperatures to register, providing enough time is allowed for temperature of the containers to become equalized with that of the surrounding air in the refrigerator. The paraffin cells have been generally used as they are more convenient to make up and handle in these experiments.

Time of Exposure necessary for Critical Temperatures to produce Mortality.

When brood material is taken from any temperature above the critical range and placed in a compartment at a lethal temperature, it requires some time for the body of the insect to chill down to the lower temperature. Larvae taken from an inactive temperature of 36° Fahr. exposed

for 2 or 3 minutes to a killing temperature of -10° F. and then brought back to active temperatures, will practically all survive. It is essential, therefore, to the technique of these experiments that the material be exposed to a critical temperature long enough for the body of the insect to chill down to this point, in order that the effect of this temperature may register.

Some attention was given to this time factor in experiments prior to 1933, and it was found that from 30 to 45 minutes of exposure at any given point was necessary before the lethal effects of the temperature were evident.

In previous experiments to determine the critical points and in most of the tests run during 1933-34, the material was kept in the cabinet and temperature lowered on a gradient of from 2 to 5° per hour. As the temperature stabilized at selected points, the material was removed and kept at room temperatures around 70° Fahr. By this method the larvae were cooled down slowly with some lag behind air temperatures in the cabinet, but received nearly a full hour of exposure either at or just above the selected point.

A series of tests were run during the winter of 1933-34 and during the fall of 1934, to determine the effect of the time factor in exposure.

Experiment No. 1, Dec. 5, 1933.

Object: To determine effect of prolonged exposure at 10° F., a temperature below the freezing point and above the initial critical point.

In previous work with D. brevicomis larvae, where short exposures of an hour or so were used, the body becomes frozen when points from 12 to 14° F. are reached. These recover when thawed out in warm temperatures, and no appreciable mortality occurs, unless they are lowered to the critical under-cooling points which begin around 2½° Fahr. 10° was selected as a point where freezing was known to occur, but still above the point where mortality had been observed in previous tests.

Material and procedure.

Mature larvae from bark collected on the Sierra Nat. Forest in November, 1933, were divided into 10 units of 50 each in paraffin cells. All 10 units were moved from room temperature into refrigerator compartments where the temperature was maintained as nearly as possible at 10° F. At each time interval shown in table 1, 2 units were removed.

Results: Table 1.

Sample No.	No. larvae	Temperature	Period of Exp.	Mortality % (48 hours after test)
Check	100	about 70	48 hours	5%
1 and 2	100	10°	14 "	9%
3 and 4	100	"	27 "	11%
5 and 6	100	"	31 "	5%
7 and 8	100	"	31 "	10%
9 and 10	100	"	39 "	1%

Discussion

In this check it is evident that mortality found in the material had no relation to time of exposure. This indicates that for temperatures just above the critical points, mortality is not induced by prolonged exposures up to 39 hours. Mortality that did occur was considered due to injury or causes other than temperature.

In previous tests allowing for exposures up to one hour, mortality higher than normal was observed to occur at 5° and just below. This appears to be the critical initial point where the less resistant individuals of the brood are killed. $2\frac{1}{2}$° was selected as a temperature just below the initial critical point and experiment 2 was run to determine whether mortality increased with prolonged exposure at this point.

Experiment No. 2, Mar. 6, 1934

Object: To determine effect of prolonged exposure at $2\frac{1}{2}$° F, a point just below the initial critical temperature.

Material and Procedure.

Mature larvae from the same source as Exp. 1 were divided into 8 lots of 50 each in paraffin cells. Methods corresponded to Exp. 1, except that compartment temperature was maintained about $2\frac{1}{2}$° F. Control was set so that temperature varied a degree or two above this point, but did not go below it. 1 unit removed at each hour interval up to 7 hours.

Results: Table 2.

Sample No.	No. larvae	Temperature	Period of Exposure	Mortality % (48 hrs. after test)
Check	50	70°	48 hours	42
1	"	$2\frac{1}{2}$	1 "	62
2	"	"	2 "	72
3	"	"	3 "	56
4	"	"	4 "	46
5	"	"	5 "	58
6	"	"	6 "	76
7	"	"	7 "	

Discussion.

These results show no positive correlation between time of exposure and mortality at 2½° F. within the range of exposure between 1 and 7 hours. Although the highest mortality was found after the longest exposure, the preceding lots show wide variation. This is considered to be due to individual variation in cold resistance at this point for larvae used in test.

In all previous tests with this species, it was found that when larvae had been chilled down to -7½° with exposures of an hour or so, the resulting mortality was 100%. It was decided therefore to determine the mortality at 10 minute intervals at this point.

Experiment No. 3, Dec. 5, 1935

Object: To determine period of exposure necessary to produce complete mortality at ultimate critical temperature of -7½° F.

Material and Methods:

Mature larvae from Sierra National Forest were divided into 14 lots of 50 each. One half of these were placed in paraffin cells, the other half in open pasteboard boxes. Lots were placed from room temperatures into cabinet with temperature maintained as near -7½° F. as possible with control. Lots of 2 were taken out at intervals shown in table 3. Control of temperature in compartment was not satisfactory and the -7½° point could not be maintained until after 30 minutes had elapsed.

Results: Table 3.

Sample Number	Number Larvae	Temperature	Period of Exposure	Mortality % (48 hrs. after test) Series A Paraffin	Series B Paper
Check	50	70°	24 hrs.	0	
"	"	"	"		0
1	"	-5	10 min.	0	
2	"	-5	"		0
3	"	-2½	20 min	10	
4	"	-2½	"		30
5	"	-6.8	30 Min	30	
6	"	-6.8	"		58
7	"	-7.7	40 Min	36	
8	"	-7.7	"		88
9	"	-7.5	50 min	46	
10	"	-7.5	"		92
11	"	-7.3	60 min	62	
12	"	-7.3	"		98

Discussion:

Results of this test are not conclusive, altho the mortality increased consistently with time of exposure. Because of stored heat in the containers, full effect of the air temperature in the cabinet was not felt by the larvae at the selected intervals and failure to reach the -7½ degree point during first part of the experiment throws considerable question on results. Mortality indicates that the paper cooled faster than the paraffin. Undoubtedly complete mortality in both lots could have occurred if exposure had been slightly longer.

With the improvement of refrigeration apparatus and electro-thermal recording during the fall of 1934, it was decided to again study the time factor in relation to mortality of western pine beetle larvae, particularly with reference to very prolonged exposures. Zero F. was selected as the point at which a series of tests would be run primarily because with short exposures at this temperature, it was found that a varying amount of mortality ranging from 10 to 80% always occured, but that complete or 100% mortality rarely if ever occurred. A constant temperature of 0 degrees F. was maintained by the University of California refrigeration plant in one of the larger rooms in Hilgard Hall, which made it possible to carry on exposures for an indefinite period at this point without any special equipment.

Experiment No. 4, November, December, 1934.

Object: To determine relation of time of exposure to mortality at 0° F. under exposures ranging from 10 minutes to 18 days.

Material and Methods:

Altogether 2800 larvae from overwintering broods collected in the field on the Lassen N. F. Oct. 25, 1934, in the 3/4 to full grown stages were divided into units of 50 each. Three separate tests were run at different periods. In two tests exposures in paraffin cells were compared with exposures in bark cells drilled in narrow plates of ponderosa pine bark and wrapped in cellophane. All material for each test was taken from 36° into the zero room and units removed at the intervals of exposure shown in the following tables. Air temperature in the room was set at 0° F. and this was occasionally checked by a mercurial thermometer Previous tests with the containers that were used indicated that about 10 minutes was required to cool down from 36° to zero and this interval was added to the exposure to allow for cooling of the larvae; i.e., the unit given 10 minutes exposure was kept in the zero room for 20 minutes. Material was kept at room temperatures of about 70° after exposure and mortality determined after 48 hours. Survivors were kept at developmental temperatures until all had either died or transformed to pupae and new adults.

Results. Table 4.

Test 4a Dec. 1 - 4, 1934

Sample Number	Number Larvae	Temperature	Period of Exposure	Mortality % (48 hours after test) Series A-paraffin cells	Series B-Bark cells
Check	100	70	48 hrs.	0%	6%
1 and 2	"	0	10 min	"	0
3 and 4	"	"	20 "	"	2
5 and 6	99	"	30 "	4	4.1
7 and 8	100	"	40 "	2	0
9 and 10	"	"	50 "	8	2
11 and 12	"	"	1 hr.	6	8
13 and 14	"	"	2 "	26	32
15	150	"	3 "	30	(no sample)
16	50	"	4 "	(no sample)	52
17	"	"	5 "	18	(no sample)
18 and 19	100	"	8 "	18	42
20 and 21	99	"	24 "	46	57.1
22 and 23	100	"	36 "	36	54
24	50	"	48 "	-	40

Test 4b Nov. 10 - 20, 1934

1	50	0	8 hrs.	28	
2	"	0	16	30	
3	"	0	24	34	
4	"	0	2 days	48	
5	47	0	3	48.9	
6	50	00	4	33	
7	"	0	5	68	
8	48	0	6	89.6	
9	49	0	7	81.7	
10	50	-	8	91	
11	50		9	78	
12	50		10	88	

Test 4c Nov. 23- Dec. 12, 1934

Check	100	0	48 hrs	0	4
1	100	0	2 days	34	40
2	100		4	64	46
3	100		6	76	50
4	100		8	74	56
5	50		11		62
6	50		12	90	
7	50		13		76
8	50		14	86	
9	50		15		66
10	49		16	83.7	
11	50		17	84	
12	50		18	40	

Discussion

A chart of results from these three tests is shown in Fig. 1. Where paraffin and bark cell containers are compared, the bark shows consistently higher mortality in test 4a, but the reverse is true in test 4c. These differences may be due to differences in conduction of the containers or altogether to chance selection of resistant individuals in the material used. Since in the majority of experiments run the paraffin cells were used, conclusions will be drawn largely from results obtained with this type of container.

These results show that with this method of exposure at 0° F. little if any mortality due to cold develops during the first 30 minutes of exposure; after 30 minutes mortality develops rapidly and within 2 hours reaches a point which does not increase materially during the first 8 hours of prolonged exposure. From 8 to 48 hours mortality increases slowly and is apparently accelerated during the 4th and 5th days of continued exposure. By the 6th day a maximum mortality is reached which does not increase with prolonged exposure. From the 6th to the 18th days of exposure at 0° Fahr. in this experiment, mortality varied from 75 to 90 percent, but these variations failed to show any relation to time. It is altogether probable that the variation in amount of mortality after the 8th day was due to chance selection of resistant individuals in each unit of material; or to this factor combined with variations in room temperature, which was held between plus 1 and -1° F.

An approximate average of mortality in relation to time as shown by the various units of material used in these tests is summarized in table 5.

Table 5. Relation of Mortality to Time of Exposure at 0° F.

Time of Exposure	Mortality %
20 min	0
30 "	5
1 hr	8
2 "	30
8 "	30
1 day	40
2 "	45
4 "	55
5 "	60
6-18 days	85

Comparison of Gradual vs. Sudden Exposure

That mortality at a given temperature increases with time of exposure is shown by results of experiment 4. Definite correlation, however, is somewhat obscured by individual variation in cold resistance and by lack of sufficient tests to determine for good averages.

In nearly all tests run to determine the killing points, the material was brought down gradually from higher to lower temperatures, thus allowing for considerable time to elapse for cooling of the body of the insect before it reaches the critical point. Where the brood is suddenly exposed to the critical point without this pre-cooling, more time is required for the temperature to take effect. It was decided, therefore, to compare those two methods by exposing two sets of material to the same temperatures under identical conditions.

Experiment No. 5

Object: To compare mortality resulting from sudden and gradual exposure to temperature ranging from plus 10 to -7½° F.

Material and Methods. Approximately 2750 larvae in the 3/4 to full grown stages were used in this experiment. These were exposed in units of 50 each in paraffin cells. The Frigidaire apparatus was used for both sudden and gradual exposure.

In the gradual exposures (test 5a) the entire group of material was removed from a temperature of 36° and placed in the refrigerator where the temperature stood at 12° F. This temperature was then lowered to 10° and sample No. 1 removed; then lowered to 7½° and sample No. 2 removed, etc. In this way temperature was brought on down to -10 degrees at the rate of about 4° per hour.

In the sudden exposures, the sample No. 1 was removed from 36° and placed in the cabinet with temperature stabilized at +10°. It was held at this point for 2 hours and sample removed; cabinet was then stabilized at 7½° and sample No. 2 exposed; and so on down to -10°. Test 5b was handled in the same way except that range of temperatures were limited from 0 to -7½°.

Results

A chart of the results from tests 5a and 5b is shown in Figure 2.

Table 6. Test 5a
Comparison of gradual and sudden exposures.

Gradual exp., series a - run Nov. 3 and 4, 1934, material collected Lassen N.F. Oct. 25, 1934
" " " b " " 17 " 18 " DO
Sudden " " Dec.30, 1934 - Jan. 1, 1933, Material collected Modoc N.F., Dec. 10, 1934

	GRADUAL EXPOSURE						SUDDEN EXPOSURE			
Temperature	Sample Number	Time Interval	Number larvae	% Mortality	Av. Mortality		2 HOURS AT EACH POINT Sample		No. larvae	Mortality %
Check at 70°	a	48 hrs	50	0.			Check at 70°		50	2
	b	"	"	4.	2.					
10°	1a		"	4.						
	1b		"	0.	2.		1		"	"
7½°	2a		"	2.						
	2b		"	24.	13.		2		"	4
5°	3a		49	8.2						
	3b		51	21.6	14.9		3		"	"
2½°	4a		50	58.						
	4b		"	34.	46.		4		"	14.3
0	5a		"	62.	62.					
	5b		"	62.	62.		5		"	10.
-2½	6a		"	58.						
	6b		"	74.	66.		6		"	20.
-5	7a		"	96.						
	7b		"	98.	97.		7		"	100.
-7½	8a		"	100.	100.					
	8b		"	100.	100.		8		"	100.
-10	9a		"	100.	100.					
	9b		"	100.	100.		9		"	100.

Test 5b
Run Jan. 7 and 8, 1935. Material collected Modoc NF. Dec. 10, 1934

Check #1:										
at 70°		48 hrs.	48	6.3		Check #2		48		0.
O.F.	1		46	33.3		1		47		36.2
-25	2		48	37.5		2		49		63.2
- 5	3		50	100.		3		50		100.
- 7½	4		"	"		4		"		"

(Check #1 counted at start of run. Check #2 removed from 36° room and counted at end of run. J.S.Y.)

Discussion

Test 5a indicates that with gradual exposure mortality resulting at the higher points within the critical range is greater than with sudden exposure, also that a point between -2½° and -5° is highly critical, and at this point mortality develops rapidly under sudden exposure. It is obvious, however, that neither method modifies the upper and lower limits of the range of killing temperatures for overwintering broods.— appreciable mortality appears at plus 5, is very high or complete at -5, and none survive -7½°. This is true for many other tests run by various methods and under differing conditions. The wide variations in mortality secured may be due as much to chance selection of resistant individuals in the samples used as to method of exposure.

The only conclusion warranted in comparing these two methods is that the ultimate range of killing temperatures will be shown either by running tests by gradual lowering of temperatures at rate of 4 or 5 degrees per hour, or by suddenly exposing samples and holding them at a stabilized temperature for a period of 2 hours.

General Conclusions

Relation of Mortality to Time of Exposure.

1. Prolonged exposure below the freezing point, but above the critical points, produces no mortality. At 10° F. no appreciable mortality was found with exposure prolonged to 39 hours. (Exp. 1.)

2. Prolonged exposures at intermediate points within the critical range definitely show that mortality increases in relation to time. An initial mortality develops within 2 hours which increases slowly during the first two days and then accelerates, reaching a maximum by the 6th day. At 0° F. this maximum mortality did not increase with exposure prolonged up to 19 days. (Exps. 2 and 4).

3. When larvae are transferred from active to critical temperatures, a precooling period is required before these temperatures take effect. With gradual lowering of temperature at rate not less than 2 to 4 degrees per hour, initial mortality occurs as soon as the critical point is reached; with sudden transfer a precooling period of 2 hours is required. (Exps. 4 and 5.)

4. With sudden transfer from active to ultimate critical temperature of -7½°, mortality is approximately complete in 1 hour. (Exp. 3)

Development of larvae surviving at 0° Fahr.

At intermediate critical temperatures certain individuals in each sample will survive while others are killed, altho the conditions

of exposure are as nearly uniform as it is possible to make them. The reason for this difference cannot be explained, but it appears to be due to an inherent resistance to cold on the part of certain larvae. However, there is a possibility that injury may occur to these survivors which is not apparent at first. To test this point the development of mature larvae which had survived varying intervals of exposure at 0° F. was followed. These larvae were kept in paraffin cells after exposure until they had either transformed to pupae and adults or died. These were kept at a constant temperature around 70° without food,- many rearing experiments having shown that food is not essential to the change of mature larvae to the prepupal condition. Selected samples which show the general run of development are given in the following table:

Table 7.

Period of Exposure at 0°F.	Survival at: 48 hours %	% which reached: pupal stage	Period required to pupate
Check	97	59	20 days
30 minutes	96	70	20
1 hour	93	71	20
1 day	48	40	30
36 hours	50	30	30
2 days	66	42	30
4 "	36	8	20
5 "	32	No development	All dead in 60 days
6 "	10.4	" "	" "
7 "	18.3	" "	" "
8 "	10	" "	" "
9 "	22	" "	" "
10 "	12	" "	" "
12 "	10	" "	" "
14 "	14	" "	" "
16 "	16.3	" "	" "
18 "	20	" "	" "
			"
			"

Considerable mortality due to method of rearing was inevitable and this is shown in the check as well as in the wide variation in the exposed samples. In the samples exposed from 30 minutes up to four days no striking differences were found in subsequent development. However, with four days' exposure there was a decisively higher mortality during subsequent development, only 8% reaching the pupal stage. After 5 days' exposure no subsequent development was discernible and all survivors died within 60 days. These results indicate that 4 days of exposure are highly critical, at 0° F. and that no development of survivors can be expected if exposure is prolonged beyond this period.

Relation of Mortality to Brood Stages

No satisfactory tests have yet been run to show cold resistance of the egg and first larval instars of the western pine beetle. This is due mainly to the fact that material in these stages is extremely difficult to handle, and samples used in experimentation develop a very high mortality from causes other than cold.

After the larvae enter the outer bark, however, and are in the 3/4 to full grown stages, they will stand a considerable amount of handling and will survive long periods without food. It is feasible to run samples either with naked larvae removed from the bark or in bark sections. Many tests have been run with larvae in these full grown stages and the critical range of temperature for overwintering broods has been fairly definitely determined as plus 5 to -7½° F.

It is considered that practically all tests run with selected larvae taken from the bark apply to the fourth or last larval instar. Salman's study of larval instars indicates that the larvae enter the outer bark during second and third instars. The third instar is very brief and larvae do not live more than 6 days without food.

Small individuals occurred in some samples used which are classed as 1/2 or less than 1/2 grown. These, however, when measured for head widths proved to be 4th instar material. They were also found to be capable of pupating without food, forming very small, runty adults. Small size is probably due to faulty nutrition.

In tests run by Yuill Nov. 3 and 4, these small individuals were classed as 1/2 grown and less than 1/2 grown and were tested separately. Results indicated that they were slightly less resistant than the full grown size at intermediate points but were killed within the same limit range of critical temperatures.

Tests have been run for pupae and new adults, and these will be presented next.

Experiment No. 6. Feb. 19 and 20, 1934

Object: To determine relative mortality of larvae and pupae of western pine beetle at critical temperatures under the same conditions of exposure.

Material: Infested bark containing mature overwintering larvae collected in the field on the Ochoco National Forest in January, 1934, was used. Part of the material was kept in warm temperatures at the Berkeley laboratory until the Majority of the brood had pupated. The remainder of the material was kept below developmental temperatures to maintain the larval state.

Methods.

Samples consisted of 450 mature larvae and 450 pupae in units of 50 each. These were taken from room temperatures and exposed in the Frigidaire compartments by gradually lowering the temperature from 10° to -10°. Within the critical range temperature was lowered at the rate of 4 to 5 degrees per hour. At each interval of 2½° one sample was removed as soon as the selected point was reached in the cabinet.

The larvae were tested on February 19, the pupae Feb. 20, 1934.

Results: Table 8

Sample	Temp. F.	No. in sample: Larvae	Pupae	Mortality 24 hrs. after exposure %: Larvae	Pupae
Check	70°	50	50	0	0
1	10	"		4	
2	7½	"		2	
3	5	"	50	14	16
4	2½	"	"	24	16
5	0	"	"	38	28
6	-2½	"	"	46	48
7	-5	"	"	70	62
8	-7½	"	"	100	100
9	-10	"	"	100	100

Discussion: Results of this test are presented in graph form in Fig. 3. Results from this material with larvae and pupae selected from the same broods and under identical conditions indicate that there is little difference in resistance of these two stages. The pupae show slightly more resistance at the intermediate points, but the range of critical temperatures and the ultimate killing point are essentially the same.

One other test was run Dec. 5, 1933, with pupae from material collected at Northfork, Calif., which gave practically the same results except that mortality was somewhat higher at critical points above zero. The surviving pupae from this test were kept at warm temperatures and these transformed to new adults except those which died from apparent injury in handling and unfavorable conditions in rearing.

Adults.

Difficulties were encountered in testing adult beetles for cold resistance which do not apply to larvae and pupae. The two immature stages are softbodied and mortality can be determined by lack of movement, and also by discoloration and changes in the body a few days after death. The adults are hard shelled and mortality can

only be detected by lack of movement at active temperatures. This is very difficult to detect particularly if the adults have been only injured by the cold.

A series of tests were made with new adults reared from infested bark during March and April, 1934, by Dr. A. Gordon.

Experiment No. 7. - March and April, 1934.

Object: To determine critical temperatures for D. brevicomis adults.

Methods and Material:

Adults reared from bark were placed in large glass vials. The number in each unit used depended upon available material at time of test, usually from 50 to 100 adults being exposed in each vial. The sudden exposure method was used. The temperature in the cabinet was first stabilized at selected points; adults were taken from room temperatures and placed in the cabinet for the determined period of exposure. They were then kept at active temperatures and studied for several days to determine the number surviving and dead. The points selected between 7 and 19° F. were separately tested as material became available from the rearing, the experiment extending through about 6 weeks.

Results: Table 9

Temp. of Exposure °F.	Sample	Number adults	Time of Exposure	Mortality following exposure- %
19°	1	100	1 hr.	0
	1a	100	18 "	0
15°	2	100	1 "	0
	2a	100	18 "	0
14°	3	100	16 "	3
	3a	26	24 "	30.8
12°	4	78	17 "	13
	4a	20	48 "	35
10°	5	100	2 "	100
	6	30	1 "	100

Discussion:

Due to difficulties mentioned above, the actual mortality was very difficult to determine, many of the beetles exposed to temperatures at 12 and 14 degrees being moribund, showing barely perceptible movement so that it was difficult to tell whether they were dead. However, the recovery of the beetles at 15° and above was quite definite as all became quite active within 1/2 hour after exposure when returned to warm temperatures. Also complete mortality at 10° was quite positive. Adults suddenly exposed to this temperature died with wings spread as though they had tried to fly before becoming chilled.

This test indicates that critical temperatures for the adults are between 15 and 10 degrees. They also indicate that mortality at intermediate killing temperatures increases with time of exposure. To test this latter point the following test was run:

Experiment 7a.

To determine relation of mortality of D. brevicomis adults to time of exposure at temperatures of 11 to 13°.

Methods and Material:

Methods were similar to experiment 6. The temperature within the cabinet was set by control so that temperatures did not fall below 11° or rise above 13° and held within these points for 100 hours. Samples were all put into cabinet and one each removed at selected intervals.

Results. Table 10

Sample	No. adults	Period of Exposure	Mortality after Exposure
1	54	2 hours	8%
2	89	3½ "	24
3	92	17 "	32
4	37	18 "	38
5	81	22 "	30
6	54	46 "	57
7	53	50 "	57
8	61	66 "	89
9	100	100 "	100

Discussions:

After 2 hours exposures moribund adults were frequent but the number of these did not increase with time of exposure. These were not considered in determining total mortality which was based upon number of individuals that were definitely determined as dead.

General Conclusions regarding critical points for adults.

While results were not altogether conclusive, the following conclusions appear to be warranted:

1. With short exposures up to 2 hours initial mortality of D. brevicomis adults appears at 14° F. and ultimate mortality at 10° F.

2. Within this range of critical temperatures mortality increases as exposure is prolonged. 100 hours of exposure produces mortality at 11 to 13° F.

Critical temperatures for First Summer Generation.

Up to the summer of 1934 all material studied for cold tests consisted of overwintering broods brought into the laboratory from the field. The work was concentrated on broods in this condition for the

reason that under field conditions only the winter broods are likely to be subjected to low lethal temperatures and the cold resistance of broods in midsummer is of little economic importance.

However, the question whether a conditioning process takes place in the fall which prepares the broods for cold winter temperatures is of considerable importance. If such is the case, a cold spell early in the fall before resistance is developed or one later in the spring after development had been resumed, would produce higher mortality than a midwinter freeze. It was decided therefore to check up on the mortality points of summer broods during the season of 1933.

Experiment No. 8. - June 1 - 4, 1934

Object: To determine points at which broods of first seasonal generation are killed by low temperatures.

Material and methods:

About 15 square feet of bark from an infested tree of the first generation were collected at Northfork, Calif., May 30, and taken by truck to the Berkeley laboratory. The brood at this time was mostly in the mature larval stages with some pupae and a few new adults. This bark material with active broods was cut into samples of 1 sq. ft. each upon arrival at Berkeley. These samples were placed at 50° over night. The following day, June 3, all except the checks were placed in the Frigidaire compartment which was lowered to 10 degrees and held at this point over night. On June 4 the compartment temperature was lowered at the rate of about 3 degrees per hour from 10 to -7° F. One sample was removed at each 2½° interval. Temperature of samples was determined by an average of 3 thermocouple points set at different positions, but all at depths of brood in outer bark. One sample was also exposed in the 0° room at Hilgard Hall for a period of 8 hours. Bark sections were cut up 4 days after exposure and the mortality was then counted.

Results: Table 11.

Sample	Temperature	No. Larvae	Mortality %
Check	70°	210	1.4
1	10°	75	6.6
2	7½°	77	5.1
3	5	152	32.3
4	2½	131	89.3
5	0	95	98.9
6	0*	145	99.3
7	-2½	94	100.
8	-5	336	100.
9	-7	105	100.

* Exposed 8 hrs. at 0 in Hilgard Hall

These results were rather surprising in that mortality

developed at higher temperatures within the critical range than for overwintering broods. Mortality was high at 5° (32.3) over 75% at 2½° and practically complete at 0.

To recheck this another test was run with material sent in from the same field locality.

Exp. 8a. June 11, 1934

Material and Methods the same as for Exp. 7, except that all samples were placed in Frigidaire cabinet, the temperature lowered to 6° F. and held at this point for 12 hours. The temperature was then lowered at the usual rate and one sample removed at each 2½ degree interval from 5° to -7. One check was held at 70° and one at 32° for a period of 24 hours each.

Results. Table 12.

Sample	Temperature °	No. Larvae:	Mortality %
Check	70°	23	4.34
Check	32	51	17.64
	5	70	51.42
	2½	78	72.36
	0	79	88.60
	0*	99	100.
	-2½	61	93.44
	-5	69	92.75
	-7	78	100.

*Exposed 8 hours in Hilgard Hall

Discussion

These tests showed the same high mortality at the higher critical temperatures as Exp. 7. However, there was greater survival at 0, -2½ and -5 degrees. In each test the mortality after 8 hours exposure in the zero room at Hilgard Hall was practically the same.

As 0° F. appeared to be highly critical for larvae of the summer broods, it was decided to use this temperature as an inde and further tests were carried out at this point. The time factor was considered in the following test:

Experiment No. 8b - June 19 to 21, 1934

Material and Methods: The same as for Exp. 7, except for that time factorx was considered in relation to constant temperature of 0° F. Naked larvae in paraffin cells were used instead of bark samples.

Results. Table 13

Sample	No. Larvae	Temperature	Time of Exposure	Mortality %
1	50	0°	2 hours	92
2	"	"	4	98
3	"	"	6	94
4	"	"	8	94

Discussion

This test, when compared with similar exposures of overwintering broods (Test 4a and 4b) shows positively higher mortality for the summer broods, particularly at shorter exposures.

Tests were made to determine whether resistance of these summer broods could be increased through conditioning before exposure. The plan adopted was to chill the larvae for short intervals, so as to simulate field conditions in the fall when cold nights and warm days are prevalent. Infested bark sections from the Northfork locality were used as samples. Exposure was made for 8 hours in the 0° room in Hilgard Hall after brood had been conditioned as shown in the following tables:

Experiment No. 8c August 11-27, 1934
Table 14.

Sample	Treatment of bark prior to exposure	Time of Exp. at 0° Fahr.	Number larvae	Mortality Percent
Check	Bark kept at room temperatures (70° to 80°) for one week, then counted	-	157	0. (50% had pupated.)
1	Kept at 70° to 80° for: 3 days; then exposed for 8 hrs. at 0°.	8 hours	104	96
2	Kept at 41° for 3 days: then exposed to 0° for 9 hours	9 "	156	98
3	Kept at 32° for 8 hrs.: then kept at 70° to 80° for 16 hrs. This alteration of temperature was repeated for 4 days; then exposed for 8 hrs. at 0°	8 "	58	86.2

Discussion

Results indicate that slight resistance was developed in sample 3 by the alternation of warm and cold temperatures, but material was not sufficient to be conclusive. Also this conditioning

process was continued only for 4 days, which may not have allowed sufficient time to develop the complete possibilities for cold hardening in the brood.

Comparison of Mortality of Winter and Summer Broods.

This series of tests indicates that for summer broods resistance to critical temperatures is perceptibly less than for winter broods; the ultimate killing point is around -2½° F. for the summer, as against -7½° for winter broods in material collected from the Northfork locality. The mortality at intermediate critical points is also higher. This can be shown by comparing the mortality at 0° for tests run under Exp. 5a, 5b.

Table 15

	Winter Broods		Summer Broods	
Mortality at 0° F. gradual exposure	49.7%	(Exp. 5a Ser. a)	98.9%	(Exp. 8)
	62	(" 5a " b)	88.6	(" 8a)
	33.3	(" 5b)		
Mortality at 0° F. Sudden exposure (2 hours)	10	(" 5a)	92	(" 8b)
	36.2	(" 5b)		

It would be expected that in the field some hardening process takes place in the fall that increases resistance of these insects to winter temperatures. However, these results do not indicate that this process materially changes the range of critical temperatures, altho the percent of kill is higher at the intermediate points.

Regional Differences in Cold Resistance of Overwintering Broods.

The distribution of the western pine beetle follows very closely that of its host, ponderosa pine. This occurs throughout a wide range of climatic conditions, varying from localities in Idaho and eastern Oregon, where minimum temperatures of -20° to -30° can be expected each winter, to the western slopes of the Sierra Nevadas in California, where temperatures as low as zero rarely occur.

It would be logical to assume that through the process of natural selection the beetles which have become acclimated to the cold localities would show greater resistance to cold than those in the warm southern localities. However, such studies as have been made on this point indicate that there is very little difference in cold resistance due to regional adaptations.

Many tests have been made during the past 6 years with overwintering broods from the Northfork locality where subzero temperatures do not occur. In all these tests, regardless of method and material used, -7½° F. was found to be the ultimate killing point and there was no survival whatever after this temperature was reached.

In the first work on this study in 1927 at Stanford University, overwintering broods from Coeur d'Alene, Idaho, were exposed in the bark and some survival was found after a temperature of -10° had been reached. These results, however, are open to considerable question, due to the inaccuracy of methods used. No further tests were made of material from cold regions until the winter of 1933-34.

During this period a series of tests were run with material from four localities representing widely different winter temperatures. These were:

1. Northfork, California. Elevation of about 3,000 ft. with prevailing winter temperatures above freezing and minimum around 10° F.
2. Modoc N. F. Elevation about 4,700 ft. with prevailing winter temperatures below freezing and minimum reaching -20° F.
3. Ochoco N. F. Elevation about 4,000 ft. with prevailing winter temperatures below freezing and minimum reaching -25° F.
4. Coeur d'Alene, N.F. Elevation about 3,000 ft. with prevailing temperatures below freezing and minimum reaching -30° F.

Experiments No. 9 - 12

Object: To determine ultimate killing temperatures of overwintering broods from different climatic conditions and relation of critical temperatures to regional conditions.

Material and Methods:

Broods collected in the field during January and February 1934 were shipped in the bark to Berkeley laboratory. As soon as practical after arrival they were exposed to critical temperatures in the frigidaire cabinet, using the method of gradual cooling. Practically all broods used were in the mature larval stages. Conditions of exposure were made as nearly uniform as possible for each lot of material. Two series were run, one with larvae removed from bark and exposed in paraffin cells; the other series with larvae in the bark.

When bark sections were used, three thermocouple points were set at depth of brood; as the selected critical points were approached, refrigeration was slowed down by control until all three points were stabilized. This gave as uniform a temperature at each point as it is possible to secure. No test was run with larvae in the bark for the

Modoc locality. For naked larvae from the Ochoco material the data from Experiment 6 was used. (Designated at Test. No. 11a in Table 16.)

Results. Table 16.

Series a. Tests run with Mature Larvae Exposed in Paraffin Cells

% Mortality & Locality

Temperature	Northfork, Calif. Test 9a: No. larvae in sample	Northfork, Calif. Test 9a: Mortality %	Modoc N.F. Test 10a: No. larvae in sample	Modoc N.F. Test 10a: Mortality %	Ochoco N. F. Test 11a: No. larvae in sample	Ochoco N. F. Test 11a: Mortality %	Coeur d'Alene Test 12a: No. larvae in sample	Coeur d'Alene Test 12a: Mortality %
Check								
70	50	0	50	2	50	0	50	0
10	"	0	"	4	"	4	"	0
7½	"	0	"	2	"	2	"	26
5	"	10	"	6	"	14	"	4
2½	"	30	"	28	"	24	"	38
0	"	38	"	20	"	38	"	20
-2½	"	40	"	34	"	46	"	48
-5	"	68	"	98	"	70	"	96
-7½	"	100	"	100	"	100	"	100
-10	"	100	"	100	"	100	"	100
-12½							"	100

Series b. Tests run with Mature Larvae in Bark Sections

	Test 9b			Test 11b		Test 12b	
Check							
70	98	6.45	No material	336	1.8	80	0
10	81	4.9	in this ser-				
7½	44	8.8	ies from	241	2.9		
5	47	10.6	Modoc N.F.	186	33.6		
2½	54	29.6		225	1.3		
0	146	23.9		163	55.3	81	
-2½	57	50.8		209	51.2	82	52.4
-5	48	78.7		167	83.9	85	96.4
-7½	49	100.		157	81.9	99	75.7
-10	79	100.		274	100.	196	90.
-12½				156	100.	116	100.
-15						130	100.
-17½						92	100.

Tests 9a, 9b, 11b, 12a and 12b are shown in graph form in Fig.4 to 8, incl.

Discussion:

No differences in the ultimate killing points were found when naked larvae were used, the material from all four localities showing 100% mortality when $-7\frac{1}{2}^{0}$ F. had been reached.

When larvae were exposed in the bark, a perceptible difference in resistance was found between the material from the northern and southern localities. In the Northfork material 100% mortality occurred at $7\frac{1}{2}^{\circ}$; in the Ochoco material there was survival at $-7\frac{1}{2}^{\circ}$ but 100% mortality at -10°; in the Coeur d'Alene material there was some survival at -10°, but mortality was complete at $-12\frac{1}{2}^{\circ}$.

Failure to thoroughly equalize the temperature at these points in all parts of each bark section, due to faulty control in the experiment might account for these differences. However, the fact that survival below $-7\frac{1}{2}^{\circ}$ occurred only in material from Northern and not from southern localities supports the results as they stand. It can be concluded therefore that for the western pine beetle, differences in cold resistance of the overwintering broods may develop, those from the more rigorous climates being hardier. However, on the basis of these tests, the regional differences appear to be slight and of only small importance in limiting mortality when critical temperatures occur in the field.

MOUNTAIN PINE BEETLE STUDIES
(G. R. Struble)

Methods of Transporting and Handling Material.

Transportation of D. monticolae broods to the laboratory was somewhat of a problem in this work. Two methods were tried throughout the experiment: (1) the shipment of naked specimens collected in the field and sent to the laboratory in thermos containers, and (2) the shipment of slabs containing brood. In the first method considerable mortality resulted even though every precaution was taken in handling and packing. The temperature conditions inside the thermos bottles were maintained several degrees below air temperature, but it was not possible to keep the temperature constant and low enough to be of advantage during the 2½ days elapsing between shipment from field to laboratory. The brood in slabs always came through in good shape, but rising temperatures in transit from field to laboratory caused activity and development which may have influenced results.

Both methods were used in shipping material from Coeur d'Alene, Idaho, and Dillon, Montana. Lodgepole broods from Crater Lake, Oregon, and all except one lot of brood from the Sierra National Forest were brought to the laboratory in slabs.

Care was taken in the laboratory tests to select only the most healthy specimens for freezing tests. Camels hair brushes were used in handling the specimens. Open cardboard boxes and paraffin cells were used as containers for the brood.

Critical Temperatures in Relation to Host.

A number of experiments have been carried out during the past six years with material from different hosts and regional localities. Overwintering broods from sugar pine in central California; lodgepole pine from northern California, southern Oregon and Montana; and western white pine from Idaho, have all been tested to determine the critical temperatures. Results have shown wide differences in the critical range and in ultimate critical temperatures, but few consistent tendencies are evident which will explain these differences. At least three factors can be assumed to have some modifying influence upon the cold resistance of this species: (1) environmental conditions due to host, (2) stages of brood development, and (3) adaptation to regional climatic conditions.

In the material that has been studied, all three of these factors interchange, particularly host and climatic influences, so that it is difficult to isolate any of them. The host factor will be considered first.

Experiment 13. Critical Temperatures in Sugar Pine.

Object: To determine the critical temperatures for overwintering larvae of D. monticolae in sugar pine.

Experiment 13a. Overwintering larvae from Sierra N.F.

Materials: Larvae in the 3/4 to fully grown stage were removed from infested slabs brought to Berkeley Nov. 1, 1933.

Procedure: Fifteen lots of 15 larvae each were placed in small pasteboard boxes and subjected to gradual cooling from 40 to 1°F. One lot was removed as temperatures reached 30°, 20°, 15°, 12°, 8½°, 6½°, 5°, 3°, 2° and 1° F. Five lots of 15 larvae each were placed at room temperature as checks. This test was conducted November 14 and 15, 1933. Mortality analyses were made November 17 and 18, 1933.

Results: Table 17

Sample #	No. Larvae	Temperature °F.	Mortality %
Check	75	70-75	29.9
1	15	30	50
2	"	20	53.3
3	"	15	40
4	"	12	26.6
5	"	8½	7
6	"	6½	100
7	"	5	78½
8	"	3	100
9	"	2	100
10	"	1	100

Experiment 13b. Overwintering larvae from Sugar Pine, Sierra N.F. (Similar to 13a as recheck of results.)

Materials: Same as experiment 13a.

Procedure: Eleven lots of 15 larvae each were placed in small pasteboard boxes and subjected to gradual cooling in the refrigerator from 25° to 3° F. All lots were removed at 1 degree intervals from 12° F. to 3° F. Five lots of 15 larvae each were placed at room temperature as checks. This test was conducted November 16, 1933. Mortality analyses were made November 18, 1933.

Results Table 18

Sample No.	No. larvae	Temperature °F.	%Mortality
Check	75	70	29.9
1	15	12	66.6
2	"	11	84.6
3	"	10	86.6
4	"	9	73.3
5	"	8	100
6	"	7	100
7	"	6	100
8	"	5	100
9	"	4	100
10	"	3	100

Experiment 13c Sugar Pine (Sierra N.F.). (This experiment similar to 13a & 13b with material collected later on during the winter.

Materials: Naked mature larvae collected from an infested log near North Fork, Calif. on Feb. 13, 1934, and brought directly to Berkeley.

Procedure: Ten lots of 50 larvae were placed in paraffin cell containers. All lots were placed in the refrigerator at 20°F. One lot at a time was removed and placed at room temperature as temperatures in the refrigerator were depressed to 15, 12, 5, 10, 7½, 5, 2½, 0, -2½ and -5 degrees F. One lot was left at room temperature as a check. The test was run from 11 A.M. to 4:45 P.M. on February 14, 1934. Mortality counts were made 24 hours after exposure.

Results: Table 19

Sample No.	No. larvae	Temperature °F.	% Mortality
Check	50	70	2
1	"	15	8
2	"	12½	90
3	"	10	100
4	"	7½	"
5	"	5	"
6	"	2½	"
7	"	0	"
8	"	-2½	"
9	"	-5	"

Discussion: These tests 13a, b and c indicate the critical range of freezing temperatures for D. monticolae in sugar pine from the central Sierra region lies between 15° F. and 8° F. Inconsistencies in results are evident and are probably due to differences in broods.

Experiment 14. Critical temperatutes in Lodge Pole Pine.
Object: To determine critical temperatures for over-wintering larvae in lodgepole pine.

Material: This brood of half to fully grown larvae was brought to Berkeley in slabs January 5, 1934, from Ft. Klamath near Crater Lake, Oregon.

Procedure: Fifteen lots of 50 larvae each were removed from slabs on January 11, 1934. These were gradually lowered in refrigeration compartments to 20, 15, 12½, 10, 7½, 5, 2½, 0, -2½, -5, -7½, -10, 12½, -15, and -17½ degrees F. one lot being taken out to room temperature as each low temperature was reached. Larvae numbering 192 were left at room temperature as checks. This test was started at 1:45 P.M. on January 11, 1934, and completed at 7:55 A.M. January 12, 1934. Final mortality analyses were made 24 hours after each exposure.

Results: Table 20

Sample No.	No. larvae	Temperature °F.	% Mortality
Check	192	70-75	0
1	50	20	0
2	"	15	0
3	"	12½	0
4	"	10	0
5	"	7½	0
6	"	5	4
7	"	2½	0
8	"	0	16
9	"	-2½	8
10	"	-5	22
11	"	-7½	52
12	"	-10	66
13	"	-12½	74
14	"	-15	94
15	"	-17½	100

Discussion: 1. Much greater resistance to cold is evident from lodgepole broods. 2. Complete mortality occurred at 17½° F. with initial killing beginning at 0° F.

A test of similar material from this same locality was run in January, 1933, which indicated a critical range from 0° --15° F. This difference between ultimate critical temperatures of -15° and -17½° may have been due to equipment as mercurial thermometers were used in the 1933 test.

Experiment 15. Critical Temperatures in Western White Pine.

Object: To determine critical temperatures for overwintering larvae in western white pine.

Materials: Naked larvae numbering 707, collected from white pine in the Coeur d'Alene National Forest, Idaho, were packed in sawdust and shipped in a thermos bottle December 19, 1933. They arrived in Berkeley, December 22, 1933, after 2½ days in transit. The larvae were sorted immediately. Fifty five or 7 percent of the total number shipped were dead upon arrival at Berkeley. All living specimens were stored at 36° F. until tests were run, beginning December 27, 1933.

Procedure: Fifteen lots of 50 larvae in pasteboard containers were subjected to gradual cooling in the refrigerator beginning at 11:30 A.M. December 27, 1933. One lot was removed as each 2½° F. lowering of temperature was reached. Lot 15 was taken out at -18° F. The first 7 lots were removed by 4:35 P.M. December 27, 1933. Lots 8-15 were left in the refrigerator over night while it warmed 10 degrees from 0° to 10° F. Temperatures were depressed December 28, 1933, from 10° to 12½° when lots 8 to 12 were removed at 2½° intervals from -2½ to -12. Lots 13 to 15 were left over night in the refrigerator while it warmed back to -2½° F. On December 29, 1933, the temperatures were again depressed and lots 13, 14 and 15 were removed as -15, -17½ and-18 degrees F. were reached. A check of 50 larvae was left at room temperature.

Results: A graph of this experiment is shown in Fig. 9.

Sample No.	No Larvae	Temperature °F.	% Mortality
Check	50	70	8
1	"	15	0
2	"	12½	0
3	"	10	4
4	"	7½	2
5	"	5	2
6	"	2½	2
7	"	0	14
8	"	-2½	18
9	"	-5½	14
10	"	-7.6	34
11	"	-10	34
12	"	-12½	60
13	"	-15	86
14	"	-17½	62
15	"	-18	92

Discussion: Host Differences (Experiments 13-15, inclusive.)
In all of these tests the material was considered to be in the overwintering stage when collected in the field. Also the conditions of exposure in the laboratory were made as nearly uniform as possible. If regional differences are not taken into account, the results show a difference in resistance of D. monticolae broods according to host.

Sugar pine broods are very much less resistant than either lodgepole or white pine broods. Experiments 12a, 13b and 13c on sugar pine brood showed critical range about the same, initial and ultimate killing points falling within very few degrees of each other. In general this range lies approximately between 12° F. and 8° F. but considerable variation is shown by high killing above 12° F., and final killing below 5°F. in experiment 13a. Similar results were obtained from sugar pine material tested in 1932. * Variations are evidently owing to differences in broods.

The critical range of overwintering broods in lodgepole and white pine as shown by experiments 14 and 15 are very much lower. A much wider gap is found between initial and ultimate killing points than in sugar pine broods. In both, initial killing started around 0° F. and final killing occurred around -17½° F. Survival of a small percent of the white pine broods subjected to -18° F. indicate even greater resistance to cold.

While it is evident that there is a wide variation in cold resistance according to host, these differences must be linked with other factors before conclusions are possible.

Critical Temperatures in Relation to Regional Localities.
The locality from which brood material is taken may be a very important factor in building cold resistant strains. While it is possible to compare the above tests according to regional localities, it must be accepted that the true relationship of critical temperatures is to an unknown extent obscurred by the host factor. Weather records of each locality from which brood material in these experiments was taken will show wide differences in winter temperature conditions. In the table which follows a comparison of host, locality, approximate winter temperature and critical range of freezing temperatures is shown.

Table 22.
Dendroctonus Monticolae Resistance
Regional Variation

Host	Locality	Approx. Low Winter Temperature ° F.	Critical Temperature range F.
Sugar pine	North Fork	25 to 10	12 to 8 (Exp.13.)
Lodgepole*	Susanville	-0 to -20	0 to -15
Lodgepole	Crater Lake	-10 to -30	0 to -17.5" 14)
White Pine	Coeur d'Alene	-15 to -30	0 to -20 ""15

*Test made November 17, 1933- reported by G.R.Struble

*Struble, G.R.-Mortality of Dendroctonus Monticolae Hopkins larvae due low temperatures. May 15, 1933. Test #3.

Discussion: Were it not for the fact that different hosts are represented, this comparison indicates that adaptations have produced more resistant strains in the more northern localities with rigorous winter climates. This point cannot bedefinitely proved, however, until comparisons are made with material from the same host tree in all localities represented.

Critical Temperatures in relation to Brood Stages.

Experiment 16a. Naked pupae. Sierra N. F.

Object: To determine the critical range of pupae in sugar pine. Pupae and adults frequently compose a portion of the overwintering broods, in some trees as high as 40 percent of the total. The effect of freezing temperatures on these two stages is an important factor in this study.

Materials and Procedure. About 10 percent of the brood in slabs used in experiment 13a were pupae. These were carefully selected and grouped into lots of 10 in pasteboard boxes. Seven lots were cooled gradually to 8, 6, 4, 2, 0, -2 and -4 degrees F. Each lot was removed to room temperature as the desired freezing temperature was reached. Five lots of 10 each were left at room temperature as checks.

Results: Table 23.

Sample No.	No. Pupae	Temperature °F.	Mortality
Check	50	70-75	14
1	10	8	50
2	"	6	10
3	"	4	50
4	"	2	50
5	"	0	100
6	"	-2	100
7	"	-4	100

Experiment 16b

Object: Comparative mortality of larvae, pupae and adults in infested blocks.

Materials and Procedure: Six infested blocks from the same lot of material as experiment 16a were cut 8" x 12" to fit the compartments of the refrigerator. One block at a time was run until the desired temperature was reached and equalized in all parts of the block. Temperature measurements were made with thermocouples and air thermometers. Blocks not run immediately were stored at 36° F. All blocks were placed in a horizontal position. Two check blocks of the same dimensions were stored at 36° F. to compare mortality results with treated blocks. Brood stages were larvae, pupae

and adults. This experiment was run between November 22 and December 12, 1933.

Results: Table 24.

Sample No.:	Exposure: Hours	Temperature: °F	Mortality Percent: Larvae	Pupae	Adults
Check	2.75	36	0	0	21
1	52½	11½	-	-	52
2	29	8½	100	55	98
3	6	8.4	100	0	94
4	2½	5½	-	-	80
5	7	4½	100	0	66
6		2	100	100	100

Discussion: (Exps. 16a, 16b.)
Although the material was inadequate for conclusions, the two experiments indicate that both pupae and adults are more resistant to freezing temperatures than larvae.

In experiment 16a fifty percent of the pupae survived at 2° F. while in experiment 16b complete mortality of pupae and adults occurred at 2° F.

In experiment 16b larval mortality was complete at 8½° F and at all temperatures below 8½° F.

Critical Temperatures in relation to Seasonal Condition.
The resistance of D. monticolae larvae to freezing temperatures may be decreased by temperatures conducive to development or increased by gradual chilling to lower temperatures. In these experiments brood materials were collected in the field during the early spring following warmer temperatures favorable to development.

Experiment 17a. Larvae from Sugar Pine. (Sierra N.F.)
Object: To determine the resistance of overwintering larvae to cold following warm spring temperatures conducive to development.

Materials: Larvae 3/4 to fully grown collected from infested slabs brought to Berkeley March 9, 1934.

Procedure: Six lots of 50 larvae were placed in paraffin cells. All lots were placed in the refrigerator at 10 A M March 15, 1934 when the temperature was 25° F. One lot was removed to room temperature at 19° F. and one at 15° F. 3 lots were removed at 14° F., 1 at 13° F. 2 at 12½° F. and 4 at 9½° F. Two lots were left at room temperature as checks. Final mortality counts were made 24 hours after each exposure. The test was completed at 4:30 P.M.

Results: Table 25.

Sample No.	No. Larvae	Temperature °F.	% Mortality
Check	100	70-75	1
1	50	19	2
2	50	15	0
3	150	14	82.66
4	50	13	78
5	100	12½	95
6	200	9½	97½

Experiment 17b Larvae from lodgepole pine,- Dillon, Montana

Object: To determine resistance of overwintering larvae, following exposure to warm temperatures.

Material: This brood was obtained from infested lodgepole slabs received at Berkeley March 26, 1934. The slabs had been exposed in transit to temperatures considerably above 50° F. and it was evident when the larvae were removed that development had started. All larvae were quite active and apparently had resumed feeding.

Procedure: Seven hundred larvae placed in paraffin cells in cans numbering 50 in each were placed in the refrigerator registering 25° F. at 5:30 on March 26, 1934. One lot of 50 was removed as each of the following temperatures was reached, and placed at room temperature: 11, 8, 7, 4.5, 3, 2½, 1, -5, -8½, -10, -12, -15, -17 degrees F. Final mortality analyses were made 24 hours after each temperature was reached. The test ended at 5:40 P.M. March 28, 1934.

Results: A graph of this experiment is shown in Fig. 10.

Table 26

Sample No.	No. Larvae	Temperature °F.	% Mortality
Check	100	70-75	3
1	50	11	-
2	"	8	8
3	"	7	28.8
4	"	4.5	63.0
5	"	3	71.4
6	"	2½	52.0
7	"	1	22.9
8	"	-5	58.3
9	"	-8.5	100
10	"	-10	--
11	"	-12	100
12	"	-15	100
13	"	-17	100

In experiment 17a, the larvae from sugar pine showed little resistance to cold temperatures below 15° F.; heavy mortality occurred at 14° F. with nearly complete mortality at 9.5° F. These results do show only a slight difference in resistance to cold compared with experiments 13a, 13b, and 13c. This is to be expected, however, since on March 9 the effect of overwintering conditions was still prevalent, even though at that time warmer temperatures had set in, causing some development.

The Dillon, Montana, material, in experiment 3b showed much less resistance to cold than lodgepole broods from Crater Lake, Oregon, or white pine broods from Coeur d'Alene, as shown by experiments 14d and 14e, even though the regional conditions are nearly the same. This indicates that seasonal changes at the time the Montana material was collected or development stimulated while the material was in transit to Berkeley, were of importance to the resistance of broods. In experiment 17b, complete mortality of larvae occurred at -8½° F., 9 degrees higher than the mortality of the Crater Lake material in the overwintering conditions.

Conclusions: Regarding Critical Temperatures for D. monticolae. (Experiments 13 - 17, inclusive.)

While it is evident that there are inconsistencies and discrepancies in the data presented, certain significant points stand out.

1. The mortality of D. monticolae larvae in all tests, regardless of host or region varied from 14° F. to -20° F., a range of 34 degrees.

2. Pupae and adult D. monticolae from sugar pine showed slightly greater resistance to cold than larvae. Further experiments on these two developmental stages is necessary as material used was inadequate.

3. There is apparent correlation between cold resistance and host when D. monticolae larvae are exposed to critical temperatures; but until further experiments are carried on with broods from different hosts in the same climatic region, the effect of host and regional influences cannot be isolated.

4. Adaptations to regional climatic conditions appear to be of importance in the cold resistance of D. monticolae larvae. Results indicate that areas such as Crater Lake, Oregon, or Coeur d'Alene, Idaho, where temperatures below zero occur annually, have developed resistant strains of larvae which can endure lower temperatures than those from the mild winter climates in Central California. The extent to which host conditions may modify this regional influence could not be determined with the material used in these experiments.

SUMMARY AND RECOMMENDATIONS

Laboratory studies dealing with low lethal temperatures affecting bark beetles have been continued at the Berkeley station during 1933 and 1934. Results of this work up to January, 1935, have been summarized and presented in this report.

The apparatus and equipment used in experimentation on this project has been further developed. Electro-thermal measurement of temperatures in refrigeration apparatus have been standardized and the accuracy of readings considerably improved over that secured in earlier experiments. The suitability of containers used for exposure of living broods to critical temperatures has been studied and the open paraffin cell selected as the best medium for this purpose.

The species studied thus far are the western pine beetle and the mountain pine beetle. These two species show wide differences in their responses to low temperatures.

WESTERN PINE BEETLE

A. Time of exposure necessary for critical temperatures to produce mortality.

This factor has been tested in a series of experiments dealing with overwintering larvae exposed to fixed temperatures for both short and prolonged intervals. For the larval material used in this series of tests, the freezing point, or crystallization of free water in the body, occurs from 14 to 12 degrees F and the range of critical temperatures which produce death is from 5 to -7½° F.

The following points were determined regarding the time factor:

1. Prolonged exposure to temperatures below the freezing point, but above the critical points did not produce mortality. At 10° F. an exposure of 39 hours resulted in no appreciable mortality.

When larvae were transferred from active to critical temperatures, the period of time necessary for the body to cool so that mortality will result was from 1 to 2 hours. When this pre-cooling was accomplished by gradual lowering of the temperature at a rate not faster than 2 to 4 degrees per hour initial mortality occurred as soon as the critical temperatures were reached. When a sudden transfer was made from active to intermediate critical temperatures, a pre-cooling

period of about 2 hours was required; at the ultimate critical temperature of -7½° F, the complete mortality resulted in approximately 1 hour.

3. At intermediate critical temperatures, the amount of mortality increased in relation to time under prolonged exposure. Initial mortality developed within the first two hours with exposure at a fixed point in the critical range; this initial mortality did not change materially during the first 8 hours of exposure; from 8 hours to 48 hours mortality increased slowly at 0° F. and from 48 hours up to 5 days mortality increased rapidly; at 0° F. a maximum mortality of 80 to 90 percent was reached by the 6th day; and with only minor variations, this maximum mortality did not change with prolonged exposures up to 18 days.

B. Development subsequent to exposure of larvae which survive critical temperatures.

1. Larvae, which survived short exposures at critical points, resumed development when brought back to active temperatures and were capable of the transformations to both the pupal and new adult stages. As the exposure was prolonged, the number of survivors which completed development was reduced. With 5 days of exposure at 0° F., no subsequent development occurred, and all survivors died before pupating; no individuals were capable of resuming development after surviving exposures ranging from 6 to 18 days.

C. Relation of Mortality to Brood Stages.

1. No satisfactory data are available as to critical temperatures affecting the egg and early larval stages.

2. The critical range for overwintering larvae and pupae was found to be practically the same for each brood stage, +5° to -7½° F.

3. Critical range for adults was found to be from 14 to 10° F.

4. Mortality of adults increased in relation to time of exposure at intermediate critical temperatures of 13° to 11° F.; 100 hours of prolonged exposure at these points resulted in complete mortality.

5. The critical range for mature larvae of the summer generation was from 5 to -5, but mortality at intermediate points was definitely higher than for overwintering larvae. For active summer larvae mortality was very high at 0° and in all but one test complete at -2½° F.

6. Resistance of summer brood larvae to intermediate critical temperatures was slightly increased by alternating daily the material between 70° and 30° F. for 4 days prior to exposure.

D. Regional Differences in Cold Resistance of Overwintering Broods.

L. Material studied from colder northern regions indicated that the ultimate critical points are somewhat lower than for material from central California, which was studied under A. B. and C. No definite differences were found for initial and intermediate critical points. The ultimate points found were: for central California -7½°; for Eastern Oregon -10°, and for northern Idaho, -12½° F.

MOUNTAIN PINE BEETLE

This species shows wide variation in critical points, ranging from 14° to -20° F. The factors responsible for this great difference between killing points have not been determined. Among those studied were:

1. Host environment.

Critical range for overwintering larvae in sugar pine was found to be from 15° to 8° F; in lodgepole pine 0° to-17½°; and in western white pine, 0 to -20°.

2. Regional adaptations.

The critical temperatures shown under hosts apply also to widely separated regional localities with different climatic conditions. These two factors cannot be isolated in the data available from experiments carried on to date. The sugar pine material was from central California, the lodgepole material from eastern Oregon, and the white pine material from Northern Idaho.

3. Brood Stages.

This factor was studied in material from the same host and locality, sugar pine in central California. Results indicate that both pupae and new adults are more resistant to cold than larvae, but material, studied was inadequate for final conclusions.

4. Seasonal Condition.

Experiments with material from sugar pine in California and lodgepole pine in Montana indicate that when overwintering larvae resume feeding and development in the spring, their resistance to cold is lessened. In sugar pine, these active larvae showed a critical range from 15° to 9½° F. as against 15° to 8° for the overwintering larvae. In lodgepole pine from Montana, active larvae showed a critical range from 7° to -8½° as against 0° to-17½° in overwintering larvae from lodgepole pine in eastern Oregon.

APPLICATION OF LABORATORY RESULTS TO FIELD CONDITIONS

The information now available regarding critical temperatures for the western pine beetle is considered to be fairly adequate in

dealing with winter kill of mature larvae, pupae and adults. It is possible to determine approximately from weather records the periods when mortality may be expected to occur in the field. Further experimentation with these brood stages seems desirable only from the standpoint of fundamental research. The data regarding critical temperatures for eggs and young larvae is insufficient, however, for practical application to field conditions.

The factors producing critical temperatures for the mountain pine beetle have not been determined in sufficient detail to warrant application of the present available information to field conditions. Results shown by these experiments are variable and to some extent inconsistent, and it will be necessary to study various factors before it will be possible to determine from weather records when mortality may be expected in the field.

RECOMMENDATIONS FOR FURTHER LABORATORY STUDIES

1. Determine the critical temperatures for the egg and young larval stages of the western pine beetle.

2. Determine the critical points for all stages of the mountain pine beetle with special reference to (a) broods in the same host tree from different regions; (b) broods in different host trees from the same region; (c) broods from the same host tree in the same region, so as to compare critical temperatures affecting both dormant and active larvae.

MANUSCRIPT REPORTS AND ARTICLES
DEALING WITH CRITICAL TEMPERATURES FOR THE
WESTERN PINE AND MOUNTAIN PINE BEETLES.

1926	Rust, H. S.	Experiment to Determine relationship of bark and air temperatures	(425.db.10)
1931	Beal, J. A.	Relation of temperature and moisture to Brood Development of the western pine beetle	(425.Db10)
	Miller, J.M.	High and low lethal temperatures for the western pine beetle	(Jour.Agr.Res. 43, 303-321)
1932	Struble, G.R.	Mortality of D. monticolae due to low temperatures	(425.Dm5)
1933	Keen, F.P.	Low temperatures as a limiting factor in western pine beetle epidemics	(425.db10)
	Salman, K.A.	Field observations on mortality of western pine beetle due to freezing	(425.db10)
	Beal,J.A.	Relation of air and bark temperatures of infested ponderosa pines during sub-zero weather	(425.db10)
	Miller, J.M.	A record of winter kill of western pine bee-tle in California	(Jour.Forestry Apr.'33-443-446)
	Gibson, A.L.	Survey of mountain pine beetle infestation on the Gallatin Nat.For. 1933	(321.g1)
		Proceedings of Forest Insect Conference Portland, Ore. Dec. 11-13, 1933	(180 F.)
1934	Beal, J. A.	Western pine beetle emergence as affected by low winter temperatures	(425.db10)
	Beal, J. A.	Relation of air and bark temperatures of infested ponderosa pines during sub-zero weather.	(Jour.Agr.Res. V.26, 133-39)
	Miller, J.M.	Effects of low temperatures during the winter of 1932-33 upon pine beetle populations in California	(Mimeographed Report)

Photo No. 1
Pasteboard box container, cover removed, showing pupae in position for exposure to critical temperatures.

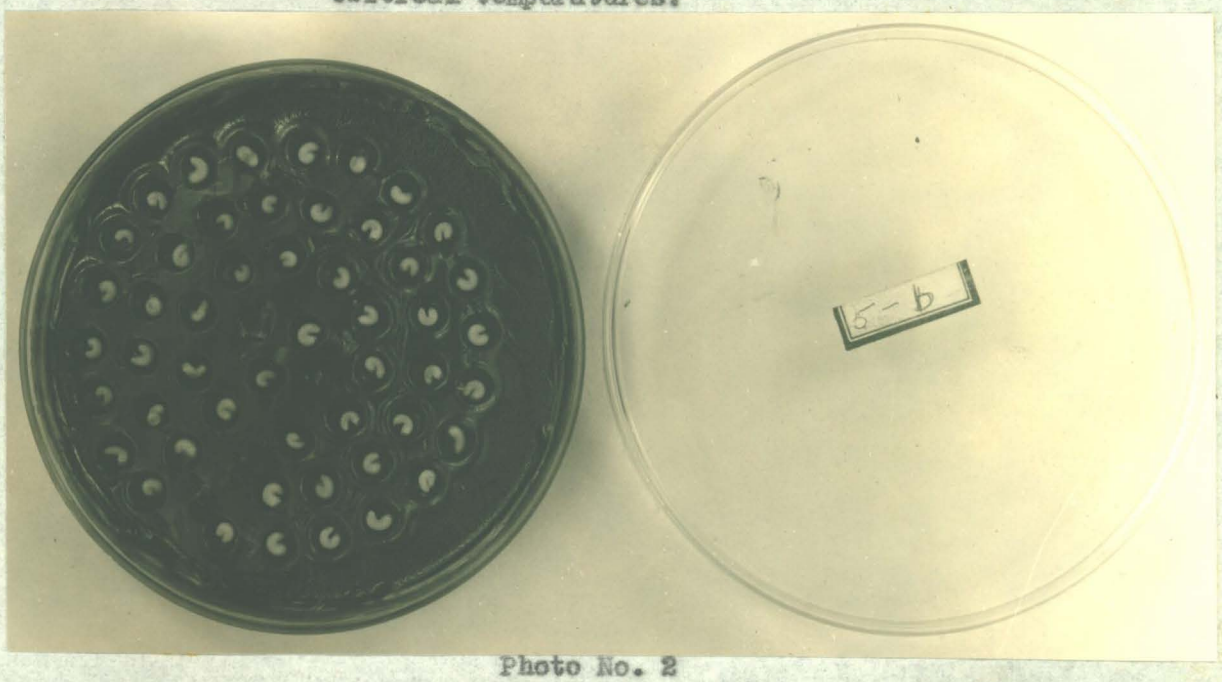
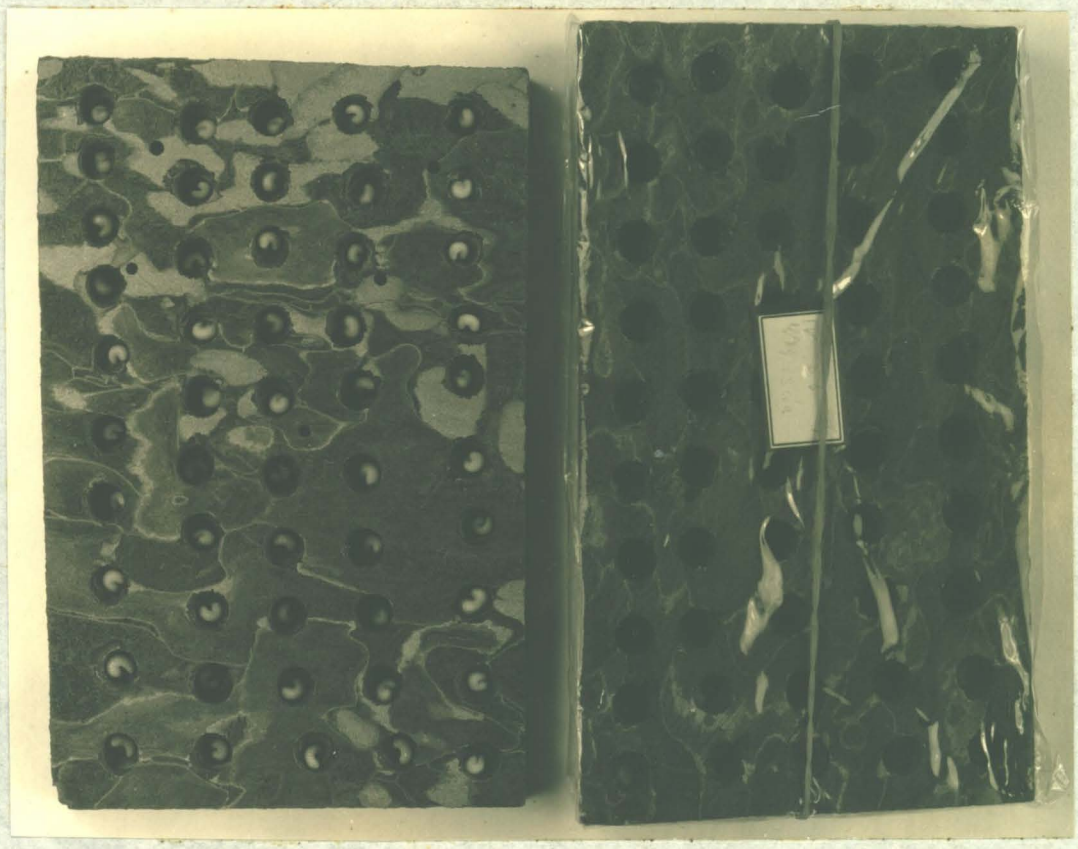


Photo No. 2
Parrafin cell container, moulded in petri dish, with cover removed, showing larvae in position for exposure to critical temperatures.



a b

Photo No. 3

Bark Cell Container Used for Exposure of Western Pine Beetle Broods to Low Temperatures.

a Unit with larvae in position

b Unit with Cellophane wrapping held in place by rubber band showing identification label

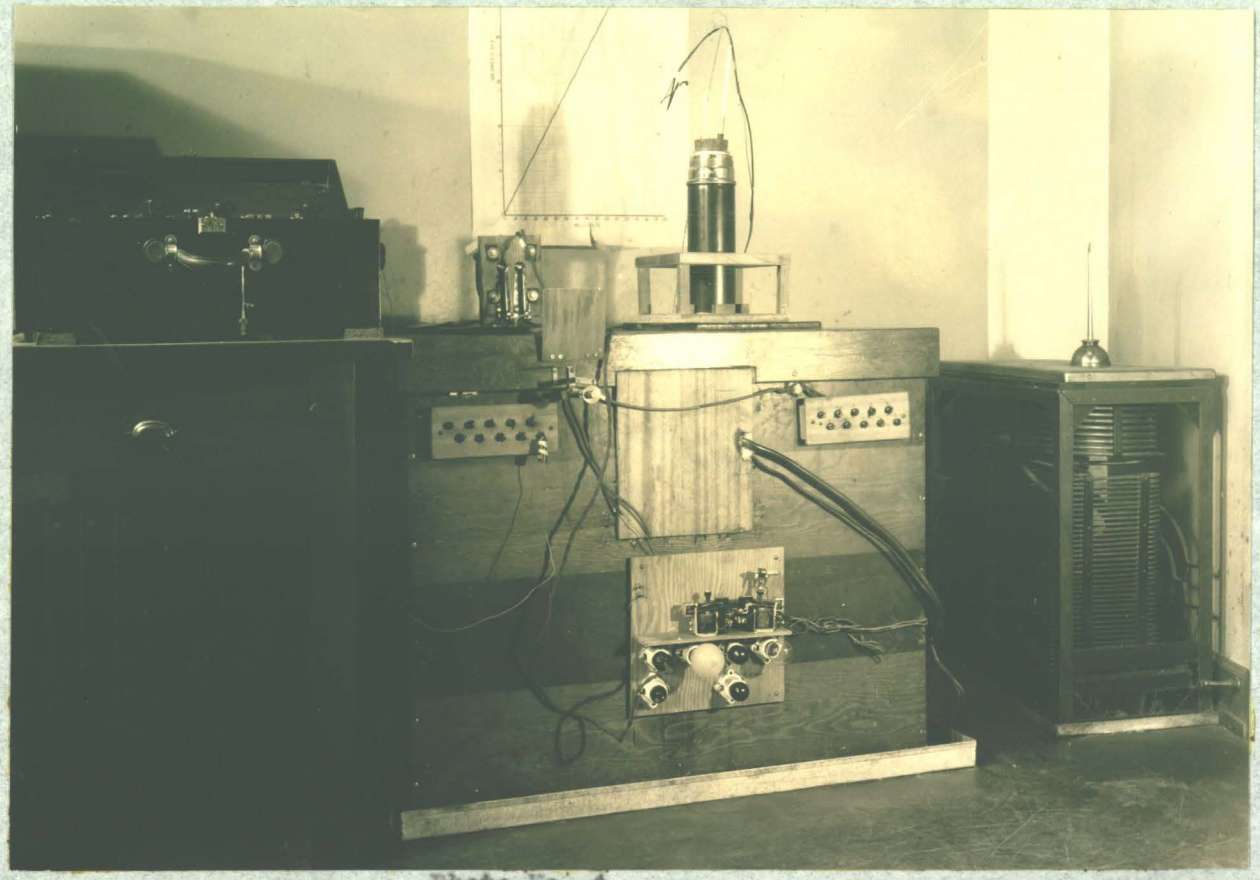


Photo No. 4

Refrigeration Unit and Electro-Thermal Equipment Developed by the Bureau of Entomology and Plant Quarantine for determining Critical Temperatures

1. Frigidaire Unit
2. Brine Cabinet
3. 3" Connections for plugging in thermo-couples.
4. Control Panel
5. Thermos bottle containing reference junction.
6. Fan mounting on lid of left compartment
7. Control device for adjusting thermo regulator
8. Potentiometer

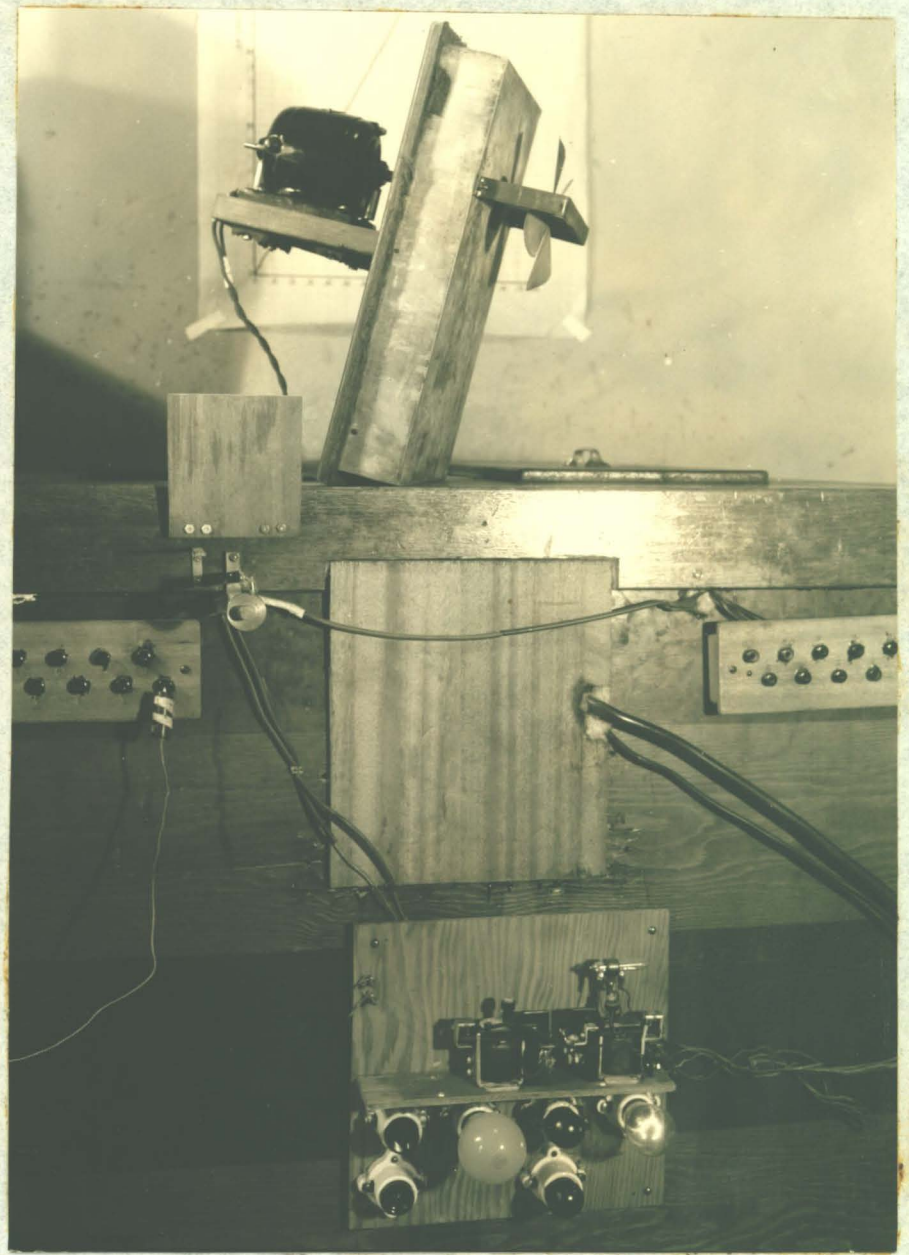


Photo No. 5

Closeup of refrigerator unit showing fan installed in lid of air compartment to break up stratification of temperatures.

1. Lid with fan
1a Motor 1/40 H.P.
1b Fan and mounting for thrust bearing
2. Control panel
2a Relay
2b Mercury switch mounted on a relay frame
2c Lamps used for resistance in thermoregulator, realy and fan circuits.

MORTALITY RESULTING FROM LOW TEMPERATURES

WESTERN PINE BEETLE FROM PONDEROSA PINE BARK FROM THE OCHOCO NATIONAL FOREST, OREGON

MATURE LARVAE AND PUPAE REMOVED FROM BARK AND EXPOSED TO LOW TEMPERATURES IN UNIT LOTS OF 50 EACH

TEST RUN AT BERKELEY, CALIFORNIA, FEB. 19-20, 1934

● Point at which brood was removed from refrigeration compartment

— Temperature in refrigeration compartment

■ Percent of mortality as determined 24 hours after removal from compartment

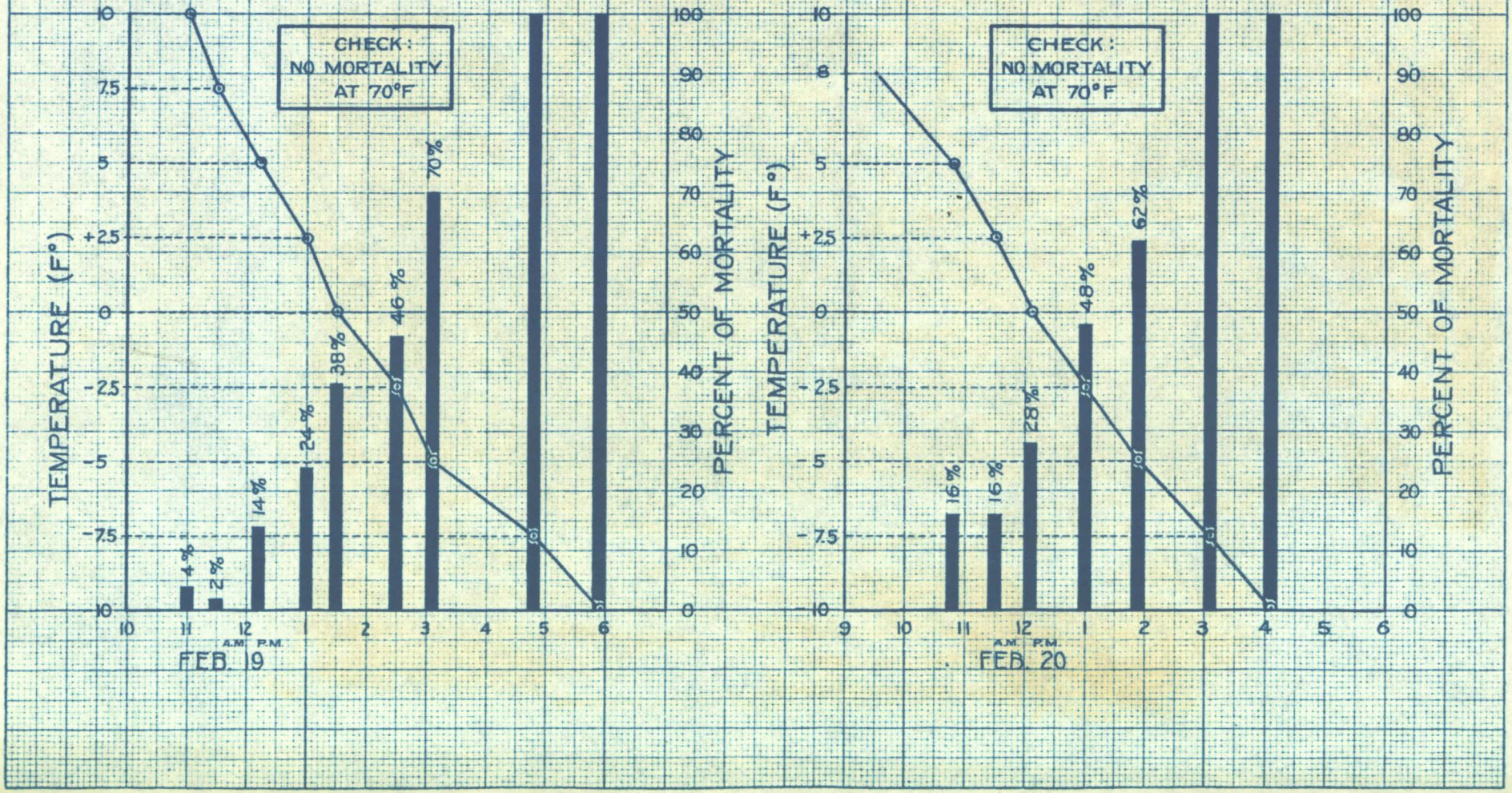


Fig 3

MORTALITY RESULTING FROM LOW TEMPERATURES

WESTERN PINE BEETLE FROM PONDEROSA PINE BARK FROM THE MODOC NATIONAL FOREST, CALIFORNIA
MATURE LARVAE REMOVED FROM BARK AND EXPOSED TO LOW TEMPERATURES IN UNIT LOTS OF 50 EACH
TEST RUN AT BERKELEY, CALIFORNIA, FEB. 12-13, 1934

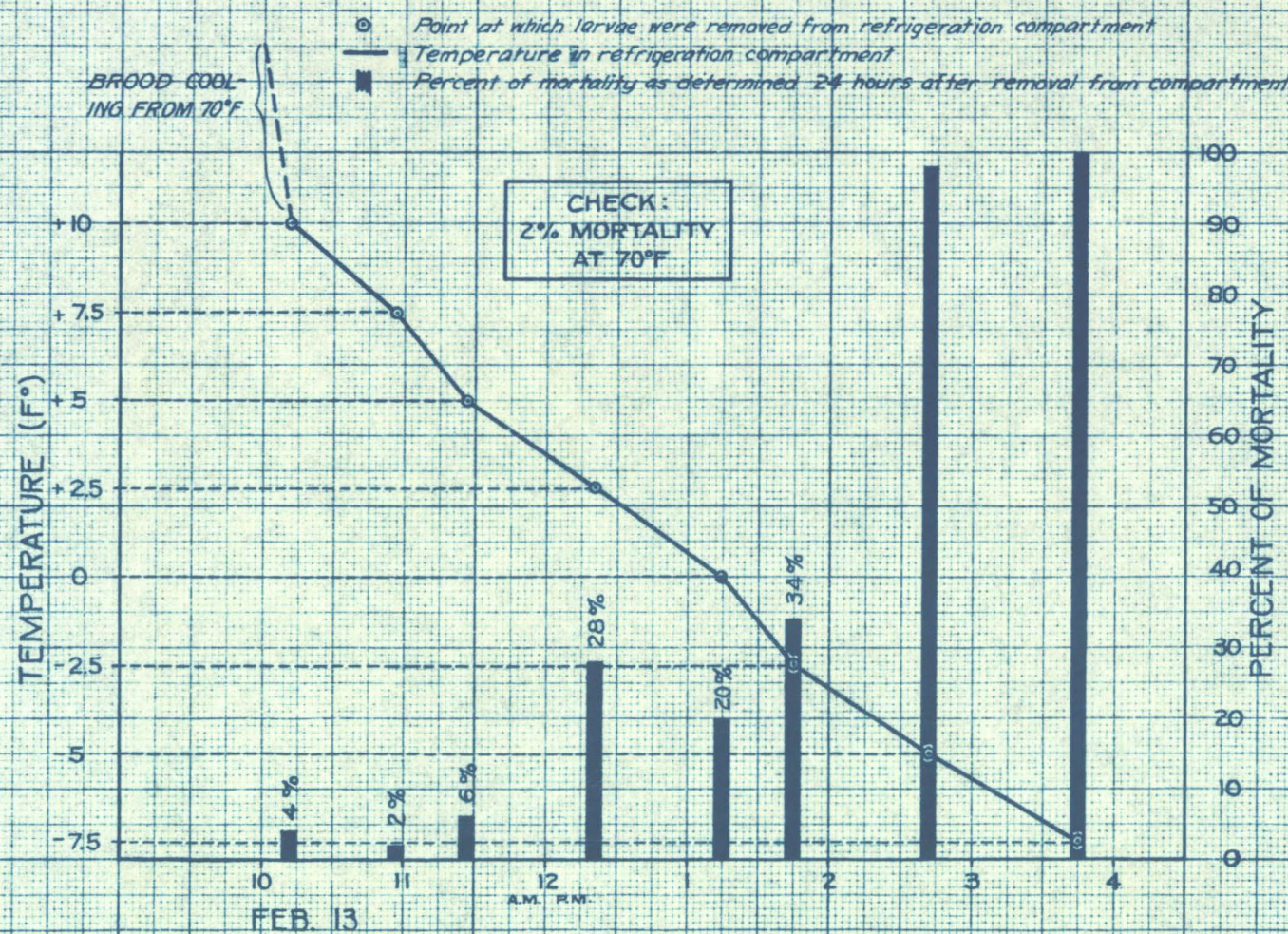


Fig. 4

KEUFFEL & ESSER CO. N. Y. NO. 359-141
Millimeters

MORTALITY RESULTING FROM LOW TEMPERATURES

MOUNTAIN PINE BEETLE IN WESTERN WHITE PINE FROM COEUR D'ALENE, IDAHO

OVERWINTERING LARVAE REMOVED FROM BACK, PACKED IN SAWDUST, AND SHIPPED TO BERKELEY, CALIFORNIA IN THERMOS BOTTLE

TEST RUN DEC. 27-29, 1933, AT BERKELEY, CALIF.

LARVAE IN UNIT LOTS OF 50 EACH IN CARDBOARD CONTAINERS

⊙ *Point at which larvae were removed from refrigeration compartment*
— *Temperature in refrigeration compartment*
▮ *Percent mortality as determined 24 hours after removal from compartment*

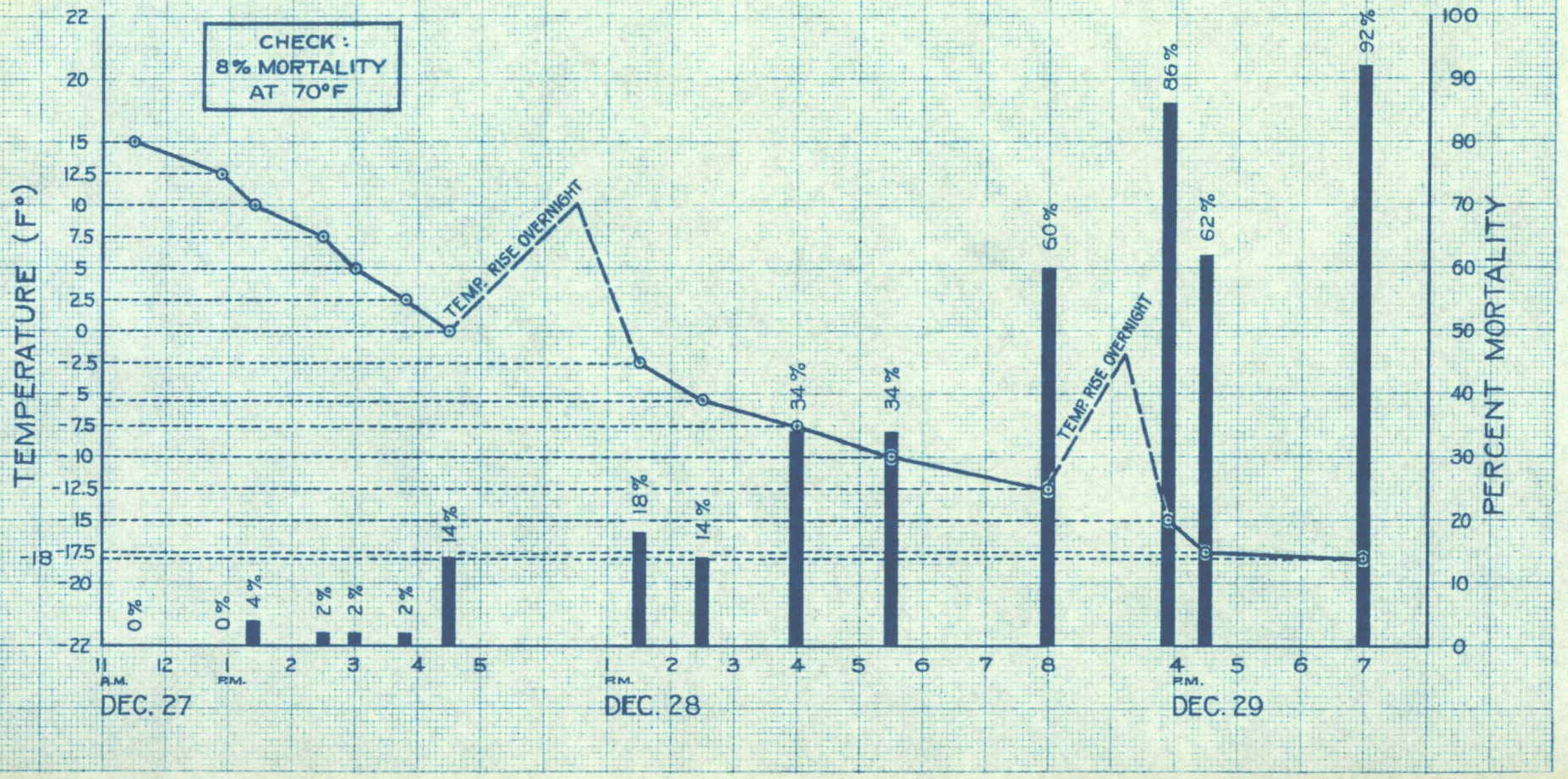


Fig. 9

MORTALITY RESULTING FROM LOW TEMPERATURES

MOUNTAIN PINE BEETLE IN LODGEPOLE PINE FROM DILLON, MONTANA

MATURE LARVAE REMOVED FROM SLABS AND EXPOSED TO LOW TEMPERATURES IN UNIT LOTS OF 50 EACH

TEST RUN MARCH 27-28, 1934, AT BERKELEY, CALIFORNIA

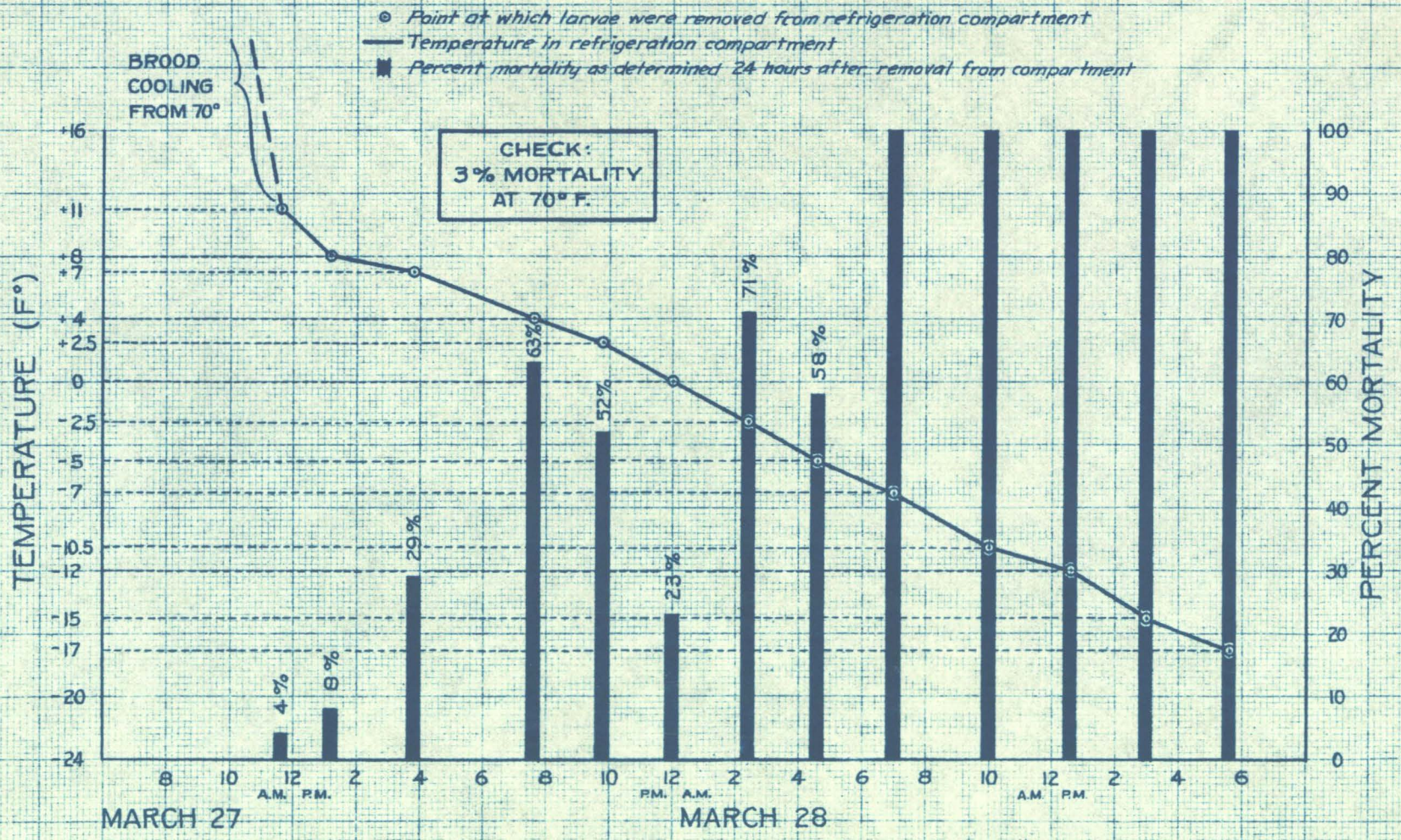


Fig. 10